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Translation No. T-712-1

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**Title:** Extraction of stimulon from cells transformed by Adenovirus 12 or Simian 40 virus (SV 40) (Extraction de stimulon de cellules transformées par l'adénovirus 12 ou par le virus simien 40 (SV 40)).

**Journal:** Compt. Rend. Acad. Sci. (Paris) 266: 2133-2135 (1968).

March 1969

STATEMENT OF WORK

This document is submitted  
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SUMMARY

Cells transformed by adenovirus 12 or Simian 40 virus contain a protein called stimulon which enhances the multiplication of rat virus K in embryonal rat cells in vitro.

Human kidney cells infected with adenovirus 12 produce a protein which stimulates the multiplication of rat virus K in embryonal cells of the rat (1, 2, 3). In the cells, adenovirus produces infectious virus and causes the lysis of the cells. The factor, designated by the name stimulon, can be extracted from the cells transformed by this virus or by the Simian 40 virus (SV 40) after a number of passages in vitro. The transformed cells do not spontaneously synthesize infectious virus. However, the persistence of the viral genome can be demonstrated by the specific T antigen of the induced virus. The technique of extraction is as follows: after removal of the culture medium, the cells are washed with 0.01 M tris-HCl buffer, pH 8, at + 4°C. They are then collected by scraping, suspended in the same buffer to a concentration of  $2 \times 10^6$  cells per ml, and disrupted by sonication (23 K cycles for 2 min). The debris is removed by centrifugation at 3,000 rpm for 5 min and the supernatant is frozen at -20°. The various extracts tested were prepared in the same fashion.

TABLE

Action of Trypsin on the biological activity of stimulon

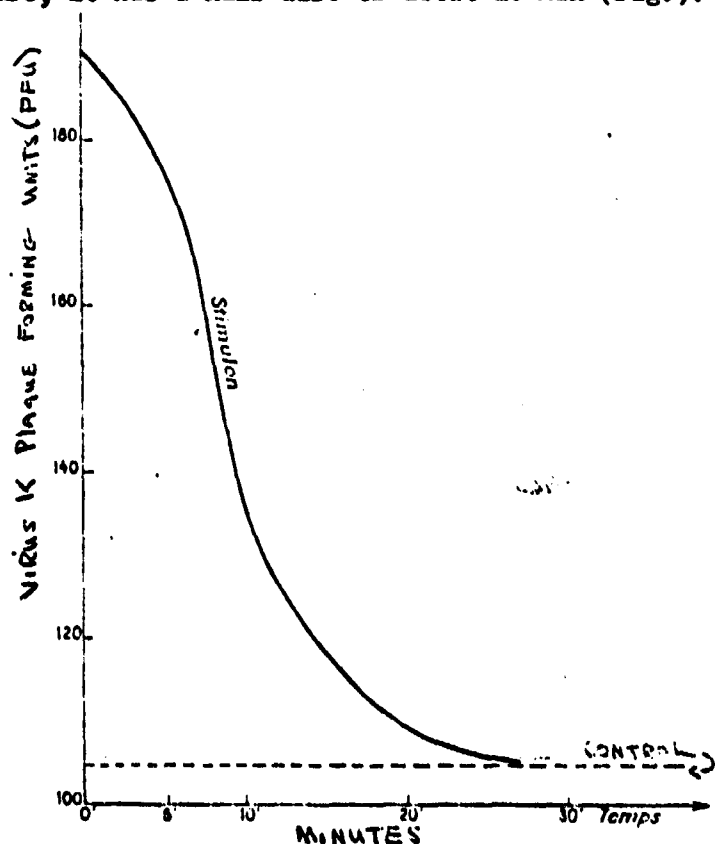
	Average number of plaques*	Range**
Control virus	105	98-115
virus K + extract	186	174-200
virus K + extracted treated with trypsin	104	97-110

\* mean value }  
\*\* range } of the number of plaques observed on 5 petri plates.

### RESULTS

The extracts obtained from cells transformed by adenovirus 12 or by SV 40, regardless of the species from which they were obtained (hamster, rat, mouse, dog, monkey) or contained thymidine kinase, increased the number of plaques produced by virus K on embryonal rat cells. Extracts prepared from normal control cells were inactive. Hamster cells transformed in vivo with methylcholanthrene or hamster cells transformed in vitro "spontaneously" under unknown conditions did not produce stimulon.

As in the lytic cycle, the stimulon extracted from cells transformed by these viruses is protein in nature. The stimulating activity of the extracts is destroyed by treatment with 2 X crystalline trypsin (200 ug at 37° for 30 min) (table). On the other hand, stimulon activity is not affected by treatment with DNase or RNase. Heating at 56° destroys the biological activity of stimulon and at this temperature, it has a half-life of about 10 min (fig.).



INACTIVATION CURVE OF STIMULON AS A FUNCTION OF TEMPERATURE (56°)

### DISCUSSION

In transformed cells, adenovirus 12 and SV 40 induce the synthesis of a protein called stimulon.

Stimulon persists in the transformed cells after several passages along with the T antigen (4,5). However, it is quite distinct from the thymidine kinase because cells transformed by SV 40 lack thymidine kinase (6) but still produce the biologically active protein.

The arguments in favor of an anti-interferon role for stimulon will be present in a subsequent paper.

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